REMARKS

The courtesy extended to Applicants' representative in the telephonic interview conducted on April 30, 2002 is gratefully acknowledged and appreciated.

Claims 1-49 are pending in the instant application. By this communication, Applicants amend claims 1 and 49. Support for the recitation of "$-NH(CH_2)_{0-12}CO\ NH(CH_2)_{0-100}NR_2R_3$" in the definition of R_1 can be found on page 6, lines 7-16 of the specification; support for an alkyl chain length of from 1-6 in the definition of R5 can be found on page 5, line 19, through page 6, line 5 of the specification.

Notwithstanding the foregoing, Applicants expressly reserve the right to prosecute subject matter no longer or not yet claimed in one or more applications that may claim priority hereto. Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and in view of the following comments.

Non-Art Related Remarks

35 U.S.C. §112, First Paragraph

The Examiner has rejected claims 1-5, 38, and 42-49 under 35 U.S.C. §112, first paragraph, indicating that the specification allegedly fails to provide support for definition of R_1 in the claims, specifically with regard to the number of methylenes "p" in "$-NH(CH_2)_pCO\ NH(CH_2)_{0-100}NR_2R_3$". As discussed with the Examiner in the telephonic interview, the specification indicates on page 6, line 16, that "p" may range from 0 to 12. Applicants, therefore, respectfully submit that the foregoing amendments to the claims render this rejection moot.

Applicants respectfully traverse the rejection of claims 2-5, 38, and 42-48 under 35 U.S.C. §112, first paragraph, contending that the structure recited in claim 2 is not within the scope of the structure recited in claim 1, from which claim 2 depends. As discussed with the Examiner in the telephonic interview, the recitation of a specific structure has been deleted from claim 2 by prior amendment. Applicants, therefore, respectfully request that the rejection be reconsidered and withdrawn.

Informalities in the Specification

The Examiner has requested that the table legend paragraph spacing presented on page 16 of the specification be corrected. Applicants submit herewith a corrected specification page as Appendix B.

CONCLUSION

Applicant believes that the present application is now in condition for allowance. Favorable consideration of the application as amended is respectfully requested. The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

Date May 6, 2002
By _____
Michael A. Whittaker
Attorney for Applicant
Registration No. 46,230

FOLEY & LARDNER
PO Box 80278
San Diego, CA 92138-0278
Telephone: (858) 847-6721
Facsimile: (858) 792-6773

Appendix A: Marked-up claims, indicating the amendments.

1. (Twice amended) A method for designing a specific polyamide

$$X_1X_2 \ldots X_m\text{-}\gamma\text{-}X_{(m+1)} \ldots X_{(2m-1)}X_{2m}\text{-}R_1$$

wherein

X_1, X_2, X_m, $X_{(m+1)}$, $X_{(2m-1)}$, and X_{2m} are carboxamide residues forming carboxamide binding pairs X_1/X_{2m}, $X_2/X_{(2M-1}$, $X_M/X_{M+1)}$,

γ is γ-aminobutyric acid or 2,4 diaminobutyric acid, and

R_1 is $-NH(CH_2)_{0\text{-}100}NR_2R_3$, $-NH(CH_2)_{[0\text{-}100]0\text{-}12}CONH(CH_2)_{0\text{-}100}NR_2R_3$, or $-NHR_2$, where R_2 and R_3 are independently selected from the group consisting of H, Cl, NO, N-acetyl, benzyl, $C_{1\text{-}100}$ alkyl, $C_{1\text{-}100}$ alkylamine, $C_{1\text{-}100}$ alkyldiamine, $C_{1\text{-}100}$ alkylcarboxylate, $C_{1\text{-}100}$ alkenyl, a $C_{1\text{-}100}$ alkynyl, and $C_{1\text{-}100}$ alkyl-L, where L is selected from the group consisting of arylboronic acids, biotins, polyhistidines comprised from about 2 to 8 amino acids, haptens, solid phase supports, oligodeoxynucleotides, N-ethylnitrosourea, fluorescein, bromoacetamide, iodoacetamide, DL-α-lipoic acid, acridine, captothesin, pyrene, mitomycin, texas red, anthracene, anthrinilic acid, avidin, DAPI, and oligodeoxynucleotide, isosulfan blue, malachite green, psoralen, ethyl red, 4-(psoraen-8-yloxy)-butyrate, tartaric acid, and (+)-α-tocopheral, suitable for use as a DNA-binding ligand that is selective for identified target DNA-sequences 5'-$WN_1N_2 \ldots N_mW$-3' where m is an integer having a value from 3 to 6, the method comprising:

(a) identifying a target sequence of double stranded DNA having the form 5'-$WN_1N_2 \ldots N_mW$-3', $N_1N_2 \ldots N_m$ being the sequence to be bound by carboxamide residues, wherein

each N is independently chosen from the group A, G, C, and T, each W is independently chosen from the group A and T, and m is an integer having a value from 3 to 6;

(b) representing the identified sequence as 5'-W*ab* ... *x*W-3', wherein *a* is a first nucleotide to be bound by the X_1 carboxamide residue, *b* is a second nucleotide to be bound by the X_2 carboxamide residue, and *x* is the corresponding nucleotide to be bound by the X_m carboxamide residue;

(c) defining *a* as A, G, C, or T to correspond to the first nucleotide to be bound by a carboxamide residue in the identified sequence;

(d) selecting Im as the X_1 carboxamide residue and Py as the X_{2m} carboxamide residue if a = G;

(e) selecting Py as the X_1 carboxamide residue and Im as the X_{2m} carboxamide residue if *a* = C;

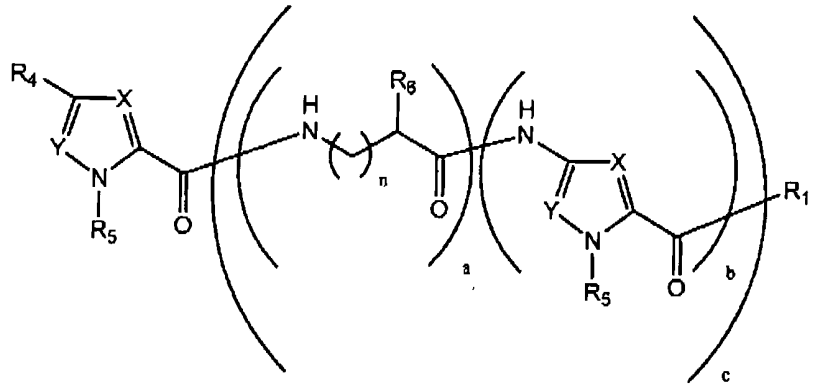
(f) selecting Hp as the X_1 carboxamide residue and Py as the X_{2m} carboxamide residue if *a* = T;

(g) selecting Py as the X_1 carboxamide residue and Hp as the X_{2m} carboxamide residue if *a* = A; and

(h) repeating steps c – g for *b* through *x* until all carboxamide residues are selected;

wherein Im is N-methylimidazole, Hp is , Py is N-methylpyrrole, A is adenine, G is guanine, C is cytosine, and T is thymine.

49. (Amended) A polyamide designed by the method of claim 1, having the structure:



wherein

R_4 is selected from the group consisting of H, NH_2, SH, Cl, Br, F, N-acetyl, and N-formyl;

each R_5 is independently selected from the group consisting of H, $(CH_2)_{0-6}CH_3$, $(CH_2)_{[0]1-6}NH_2$, $(CH_2)_{[0]1-6}SH$, $(CH_2)_{[0]1-6}OH$, $(CH_2)_{[0]1-6}N(R_7)_2$, $(CH_2)_{[0]1-6}OR_7$, and $(CH_2)_{[0]1-6}SR_7$, wherein R_7 is $(CH_2)_{0-6}CH_3$, $(CH_2)_{[0]1-6}NH_2$, $(CH_2)_{[0]1-6}SH$, or $(CH_2)_{[0]1-6}OH$;

each R_6 is independently selected from the group consisting of H, NH_2, OH, SH, Br, Cl, F, OMe, CH_2OH, CH_2SH, and CH_2NH_2;

R_1 is $-NH(CH_2)_{0-100}NR_2R_3$, $-NH(CH_2)_{[0-100]0-12}CONH(CH_2)_{0-100}NR_2R_3$, or $-NHR_2$, where R_2 and R_3 are independently selected from the group consisting of H, Cl, NO, N-acetyl, benzyl, C_{1-100} alkyl, C_{1-100} alkylamine, C_{1-100} alkyldiamine, C_{1-100} alkylcarboxylate, C_{1-100} alkenyl, a C_{1-100} alkynyl, and C_{1-100} alkyl-L, where L is selected from the group consisting of arylboronic acids, biotins, polyhistidines comprised from about 2 to 8 amino acids, haptens, solid phase supports, oligodeoxynucleotides, N-ethylnitrosourea, fluorescein, bromoacetamide, iodoacetamide, DL-α-

lipoic acid, acridine, captothesin, pyrene, mitomycin, texas red, anthracene, anthrinilic acid, avidin, DAPI, and oligodeoxynucleotide, isosulfan blue, malachite green, psoralen, ethyl red, 4-(psoraen-8-yloxy)-butyrate, tartaric acid, and (+)-α-tocopheral;

each X and Y are independently selected from the group consisting of N, CH, COH, CCH_3, CNH_2, CCl, and CF;

each n is an integer from 1 to 2;

each a is an integer from 0 [or] to 1;

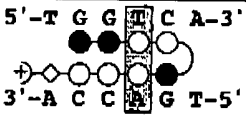
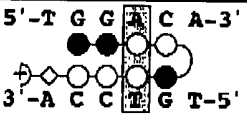
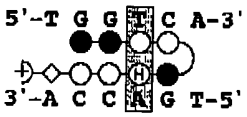
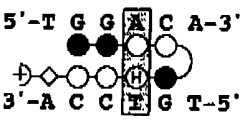
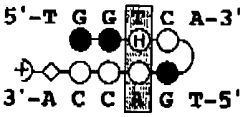
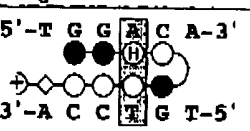
each b is an integer from 1 to 5; and

c is an integer [value] from 2 to 10.

Appendix B: Replacement specification pages 16 and 16a

181 (1986); The K_d values were determined by quantitative DNase I footprint titration experiments: on a 3' ^{32}P-labeled 250-bp DNA fragment containing the target sites, 5'-TGGACA-3' and 5'-TGGTCA-3' which differ by a single A•T base pair in the fourth position. The DNase footprint gels are shown in Figure 3.

TABLE 1 Equilibrium dissociation constants*

	Polyamide†	5'-TGGTCA-3'	5'-TGGACA-3'	K_{rel}‡
1	Py/Py	 K_d = 0.077 nM	 K_d = 0.15 nM	2.0
2	Py/Hp	 K_d = 15 nM	 K_d = 0.83 nM	0.06
3	Hp/Py	 K_d = 0.48 nM	 K_d =37 nM	77

* The reported dissociation constants are the average values obtained from three Dnase I footprint titration experiments. The standard deviation for each data set is less than 15% of the reported number. Assays were carried out in the presence of 10 mM Tris·HCl, 10 mM KCl, 10 mM $MgCl_2$, and 5 mM $CaCl_2$ at pH 7.0 and 22°C.

† Ring pairing opposite T·A and A·T in the fourth position.

‡ Calculated as K_d(5'-TGGACA-3')/K_d(5'-TGGTCA-3').

Based on the pairing rules for polyamide-DNA complexes both of these sequences are a match for control polyamide **1** which places a Py/Py pairing opposite A•T and T•A at both sites. It was determined that polyamide **1** (Py/Py) binds to 5'-TGGTCA-3' and 5'-TGGACA-3' within a factor of 2 (K_d = 0.077 or 0.15 nM respectively). In contrast, polyamide **2** (Py/Hp) binds to 5'-TGGTCA-3' and 5'-TGGACA-3' with dissociation constants which differ by a factor of 18 (K_d = 15 nM and 0.83 nM respectively). By reversing the pairing in polyamide **3** (Hp/Py) the dissociation constants differ again in the opposite direction by a factor of 77 (K_D = 0.48 nM and

37 nM respectively). Control experiments performed on separate DNA fragments; reveal that neither a 5'-TGGGCA-3' or a 5'-TGGCCA-3' site is bound by polyamide **2** or **3** at concentrations ≤ 100 nM, indicating that the Hp/Py and Py/Hp ring pairings do not bind opposite G•C or C•G.

The specificity of polyamides **2** and **3** for sites which differ by a single A•T/T•A base pair results from small chemical changes. Replacing the Py/Py pair in **1** with a Py/Hp pairing as in **2**, a single substitution of C3-OH for C3-H, destabilizes interaction with 5'-TGGTCA-3' by 191-fold, a free energy difference of 3.1 kcal mol^{-1}. Interaction of **2** with 5'-TGGACA-3' is destabilized only 6-fold relative to **1**, a free energy difference of 1.1 kcal mol^{-1}. Similarly,